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Phytochemical characterization, antioxidant and antidiarrheal activities, and safety profile of Lomariopsis japurensis rhizome extract

Sandra Liliana Hernández-Salón

sandrasalon2000@gmail.com

Universidad Internacional de las Américas https://orcid.org/0000-0001-6049-1639

Fabián Delgado-Rodríguez

Universidad de Costa Rica

Nien Tzu Weng

Universidad de Costa Rica

Sara González

Universidad de Costa Rica

Olman Hidalgo

Universidad de Costa Rica

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Phytochemical characterization, antioxidant and antidiarrheal activities, and safety profile of *Lomariopsis japurensis* rhizome extract

Sandra Liliana Hernández-Salón[1,2*], Fabián Delgado-Rodríguez[3], Nien Tzu Weng[3], Sara González[2], Olman Hidalgo[3]

1. Universidad Internacional de las Américas, San José, Postal code: 10101, Costa Rica
2. LEBi, Universidad de Costa Rica, San José, Postal code: 11501-2060, Costa Rica
3. Institute for Pharmaceutical Research (INIFAR), Faculty of Pharmacy, Universidad de Costa Rica, San José 11501-2060, Costa Rica

*Corresponding author: sandrasalon2000@gmail.com

Abstract

Background: *Lomariopsis japurensis* is a fern traditionally used by Indigenous Amazonian communities for gastrointestinal disorders, including diarrhea; however, experimental evidence supporting this use remains limited. This study evaluated the phytochemical screening, *in vitro* antioxidant properties, antispasmodic and antidiarrheal activities, and acute oral toxicity of *L. japurensis* rhizome aqueous extract.

Results: Phytochemical screening revealed flavonoids, tannins, coumarins, saponins, triterpenoids, and sterols, with high total phenolic and flavonoid contents. The extract exhibited antioxidant activity in different *in vitro* assays. In isolated mouse ileum, *L. japurensis* rhizome extract exhibited weak intrinsic contractile activity but reduced responsiveness to acetylcholine. Preincubation with 1.0 mg/mL *L. japurensis* rhizome extract significantly increased the acetylcholine EC_{50} from 4.65 ± 1.92 to 20.15 ± 5.77 nM, indicating decreased sensitivity to cholinergic stimulation. *In vivo*, *L. japurensis* rhizome extract (2,000 mg/kg) reduced gastrointestinal transit from 71.30 ± 4.87% in the control group to 59.28 ± 3.32%, corresponding to a 16.9% inhibition. LJRE also reduced liquid stools from 3.4 ± 1.0 to 1.0 ± 0.55 in the castor oil-induced diarrhea model, corresponding to a 70.6% reduction. No mortality, treatment-related clinical signs, or macroscopic abnormalities were observed following acute oral administration at

doses up to 2,000 mg/kg. Conclusions: The present study with LJRE showed antispasmodic and antidiarrheal effects, together with *in vitro* antioxidant activity and a low acute oral toxicity profile under the experimental conditions evaluated. The marked reduction in liquid stools relative to the more moderate inhibition of gastrointestinal transit suggests that its antidiarrheal activity may involve mechanisms beyond reduced intestinal propulsion. These findings provide experimental support for the traditional use of LJRE for diarrhea and warrant further studies to identify its bioactive constituents and clarify their mechanisms of action.

Key words: *Lomariopsis japurensis*, Antidiarrheal activity, Antispasmodic activity, Intestinal motility, Phytochemical screening, antioxidant activity, medicinal ferns.

Background

Diarrheal diseases continue to represent a major public health concern, particularly in developing countries, where they remain one of the leading causes of morbidity and mortality among children under five years of age and older adults. According to the World Health Organization, diarrheal infections are responsible for approximately 443,832 deaths annually among children under the age of five and 50,851 deaths among children aged 5–9 years. In addition, they constitute one of the leading causes of childhood malnutrition (WHO 2024).

The high incidence of diarrheal diseases is associated with several social and environmental determinants, including limited access to safe drinking water, inadequate sanitation, malnutrition, and insufficient access to healthcare services. These factors facilitate pathogen transmission and increase the severity of diarrheal illnesses (Chari et al. 2023). The disease burden is also considerable among adults. In the United States, it is estimated that on average every adult experiences at least one episode of infectious diarrhea each year. Of these cases, approximately 500,000 require hospitalization, and more than 5,000 result in death, with older adults presenting the highest mortality risk. In addition to their impact on health, these infections impose substantial economic costs associated with hospital care and loss of work productivity (Meisenheimer et al. 2022).

From a clinical perspective, diarrhea may arise from both infectious and noninfectious causes. Noninfectious diarrhea can be associated with functional gastrointestinal disorders, food intolerances, medication-related adverse effects, and inflammatory conditions. These disorders may involve alterations in intestinal motility, increased stool frequency, abdominal discomfort, and spasmodic pain, which can substantially impair

patients´ quality of life. Moreover, gastrointestinal spasms and altered intestinal contractility are not exclusive to diarrheal conditions but may also occur in other gastrointestinal disorders, highlighting the potential therapeutic relevance of agents with antispasmodic properties. Although oral rehydration remains essential for preventing dehydration and specific treatments are required according to the underlying etiology, additional therapeutic approaches aimed at modulating intestinal motility and alleviating gastrointestinal spasms may provide symptomatic benefits in selected clinical conditions. However, the use of antimotility agents in infectious diarrhea requires careful consideration because they may be contraindicated in certain invasive or inflammatory infections. (Chavda et al. 2024; Meisenheimer, 2022).

Ethnobotany constitutes an important source of knowledge regarding medicinal plants traditionally used for the treatment of gastrointestinal disorders. For generations, communities from different cultures have employed plant extracts to relieve intestinal spasms, abdominal pain, and other symptoms associated with diarrhea. Scientific validation of this traditional knowledge may contribute to the identification of bioactive compounds with therapeutic potential and provide evidence supporting their safe incorporation into evidence-based medical practice (Liu et al. 2024).

The pharmacological investigation of medicinal plants with antispasmodic activity involves both the phytochemical identification of bioactive metabolites and the experimental evaluation of their effects on intestinal motility using *ex vivo* and *in vivo* models. The integration of these approaches allows the relationship between phytochemical composition and biological activity to be established, thereby providing mechanistic evidence regarding their potential therapeutic effects (Akinmurele et al. 2023).

Although most research has focused on species belonging to the division Magnoliophyta (angiosperms), other plant groups have received considerably less attention. Among these are ferns, which represent a promising source of secondary metabolites with biological activities that remain largely unexplored (Moussa et al. 2024). One such species is *Lomariopsis japurensis*, which has traditionally been used by several indigenous communities of the Amazon to treat a variety of conditions, including disorders related to pregnancy and childbirth, menstrual pain, liver diseases, stomach pain (Navarrete et al. 2006), and diarrhea (Odonne et al. 2013). Despite these traditional uses, the pharmacological properties of *L. japurensis* have been scarcely investigated, and its phytochemical composition and safety remain largely unexplored.

Therefore, the present study aimed to experimentally evaluate the traditional antidiarrheal use of *L. japurensis* by assessing its antispasmodic activity using *ex vivo* organ bath experiments and an *in vivo* gastrointestinal transit model, as well as its antidiarrheal activity in a castor oil-induced diarrhea model. The study also included phytochemical screening, evaluation of antioxidant activity, and acute oral toxicity assessment to provide an integrated characterization of the pharmacological properties and safety of the extract.

Methods

Study materials and design

Fresh rhizomes of *Lomariopsis japurensis* were collected from the Surá trail at La Selva Biological Station, Costa Rica, on February 2, 2018. A total of 1 kg of fresh material was harvested following prior population assessment conducted by the Organization for Tropical Studies within La Selva Biological Reserve. Collection was conducted under authorization granted by the National System of Conservation Areas (SINAC), Costa Rica, under scientific collection permit No. SINAC-ACC-019-2018. All procedures were carried out in accordance with national biodiversity conservation regulations and applicable environmental legislation.

A representative voucher specimen was deposited at the National Herbarium of Costa Rica under catalog number CR-296382. Taxonomic identification was performed by Dr. Alexander Rojas Alvarado (Universidad Nacional, Costa Rica), specialist in Pteridophytes.

Approximately 1 kg of rhizome was processed. Plant material was dried at approximately 45 °C in a custom-built drying chamber at the Institute for Pharmaceutical Research (INIFAR). Prior to milling, the dried material was manually fragmented. Grinding was performed using a Wiley mill to obtain a homogeneous powder.

The dried and powdered material was subjected to aqueous decoction using a plant material-to-solvent ratio of 1:10 (w/v). In the absence of documented ethnopharmacological preparation times for this species, a 20-minute decoction period was selected based on historical preparation protocols described for male fern (Raspail, 1853). The decoctions were concentrated under reduced pressure using a Büchi R-100 rotary evaporator equipped with a Büchi B-00 water bath. The concentrated extracts were transferred to lyophilization flasks and frozen at approximately −70 °C. Freeze-drying was carried out for 4 days using a

Labconco FreeZone 4.5 lyophilizer. The lyophilized extracts were weighed and stored at −20 °C until further analysis.

Experimental procedures

1. Detection of secondary metabolites groups by high-performance thin layer chromatography (HPTLC):

1.1 General experimental conditions:

Extract solution (20 mg/mL) was prepared in water, filtered through nylon filters with 0.45 µm pore size and transferred to autosampler vials. From each extract, 25 µL were applied on HPTLC grade 60 F254 silica gel plates (20 x 10 cm) (Millipore, Germany). The samples were applied in the form of bands using spray mode, nitrogen was used as propellent gas (application speed 150 nL/s). Bands dimensions were fixed to 8x1 mm using the ATS4 autosampler (CAMAG, Switzerland). The distance between the left border of the plates and the first band was 2 cm. The plates were dried for 10 minutes at room temperature and developed in a conventional glass TLC chamber (Shandon TLC Chromatotank, England) previously saturated with mobile phase by 20 minutes. The solvent front position was fixed at 8 cm from the plate bottom. After development, plates were visualized under visible or ultraviolet light (UV) (366 nm wavelength) using the TLC Visualizer (CAMAG, Switzerland).

1.2. Preliminary phytochemical screening by TLC

1.2.1 Detection of phenolic compounds:

The development system for the detection of phenolic compounds was a single elution step using the mobile phase butan-2-ol: n-butanol: ethyl acetate: formic acid (60:40:15:10 v/v/v/v). After the development of plates, they were derivatized with potassium ferricyanide and ferric chloride reagents (general detection of phenolics), aluminum chloride reagent (detection of flavonoids) or potassium hydroxide solution (detection of coumarins, flavonoids, and quinones). All reagents were prepared and applied according to Lock de Ugaz (1994). Phenolic compounds produce blue stains after their reactions with potassium ferricyanide and ferric chloride reagents. Reaction of quinones with potassium hydroxide turns them red. Aluminum chloride reagent promotes the generation of complexes with flavonoids that intensifies their fluorescence under UV light (366 nm wavelength) (Lock de Ugaz, 1994). Exposition of flavonoids to alkali solutions increases their yellow color

or turns them brown, reddish, or orange (Ahluwalia et al. 1958). The fluorescence intensity of coumarins observed under UV light (366 nm wavelength) is increased by their exposition to potassium hydroxide (Lock de Ugaz, 1994).

1.2.2 Detection of terpenoids:

The detection of terpenoids was performed using the mobile phase chloroform: methanol: water (100:40:5 v/v/v). After development of plates, they were derivatized with anisaldehyde/sulfuric acid reagent or Liebermann-Burchard's reagent. These reagents were prepared and applied according to Lock de Ugaz (1994). The exposition of terpenoids (monoterpenes, sesquiterpenes, triterpenes and steroids) to anisaldehyde/sulfuric acid reagent produces blue, violet, gray, brown, or reddish colors under visible light (Agatonovic-Kustrin et al. 2019, Lock de Ugaz 1994). Triterpenes and sterols react with Liberman-Burchard's reagent giving green, yellow, brown, violet, or reddish colors when the plate is observed under visible light (Lock de Ugaz 1994, Pascual et al. 2002, Wutsqa et al. 2021).

1.2.3 Detection of alkaloids:

The detection of alkaloids was performed with the mobile phase chloroform: methanol: ammonium hydroxide 30 % w/w (47.5:47.5:5 v/v/v). After plate development, they were derivatized with Dragendorff's reagent according to Lock de Ugaz (1994). Exposition of alkaloids to Dragendorff's reagent turns them into orange or reddish stains (Lock de Ugaz 1994, Zhang et al. 2021).

1.3. Additional tests for the phytochemical screening:

In addition to HPTLC analysis, additional qualitative phytochemical tests were performed to assess secondary metabolite classes not evaluated by the chromatographic approach. Saponins were evaluated using the foam test, whereas tannins were assessed using ferric chloride and gelatin–salt tests. Positive reactions were determined according to the characteristic response of each assay corresponding to persistent foam after agitation with water, dark blue coloration, and white precipitates, respectively (Lock de Ugaz 1994; Dubale et al. 2023)

1.4. Quantification of phenolic compounds:

1.4.1 Determination of Total Phenolic Content (TPC):

TPC value of the extract was determined according to the Folin-Ciocalteu method described by Bobo-García et

159 al. (2015). The determination of TPC for LJRE was carried out in triplicate. TPC value is reported as the average
160 concentration of gallic acid equivalents (GAE) (mg GAE/g of extract dried weight (DW)) with the
161 corresponding standard error of the mean (SEM).

162 1.4.2 Determination of Total Flavonoid Content (TFC):

163 Aluminum chloride method reported by Magalhães et al. (2012) was used for the quantification of TFC values
164 of the extract with modifications. Quercetin was used as a reference flavonoid at a concentration range of 20-
165 60 µg/mL. The determination of TFC for LJRE was carried out in triplicate. TFC value is reported as the average
166 concentration of quercetin equivalents (QE) (mg QE/g DW) with the corresponding SEM.

167 1.5 Evaluation of antioxidant activity:

168 1.5.1. Oxygen Radical Absorbance Capacity assay (ORAC):

169 ORAC value of LJRE was determined according to the method described by Kenny, Brunton, and Smyth (2015)
170 with modifications. Fluorescence decay curves for the standard and the sample wells were monitored for 1
171 hour. The trolox calibration curve was built with a linearity range of 20-60 µM. The ORAC value of LJRE was
172 measured in triplicate and the antioxidant activity is reported as the average concentration of trolox
173 equivalents (TE) (mmol TE/g of extract DW) with the corresponding SEM.

174 1.5.2. Ferric Reducing Antioxidant Power assay (FRAP):

175 The assessment of FRAP value of the extracts was determined by the adaption of the assay described by Işıl et
176 al. (2010) to 96-wells microplate format. Trolox standard solutions were prepared in methanol with a linearity
177 range of 20-100 µM. LJRE solution was prepared with an adequate concentration for the determination of their
178 antioxidant activity by interpolation in the trolox calibration curve. Aliquots of 20 µL of trolox or LJRE solutions
179 were dispensed in the wells of the microplate. Then, the following reagents were added in order: 130 µL of 30
180 mM HCl, 25 µL of 1.2 % w/v potassium ferricyanide and 25 µL of 0.1 % w/v iron (III) chloride hexahydrate. As
181 blank for the standard, 20 µL of methanol was used instead of trolox and the procedure was applied as
182 mentioned before. For extracts, the blank was 20 µL of the corresponding solution treated with the same
183 reagents, substituting the 25 µL aliquot of 1.2 % w/v potassium ferricyanide by 25 µL of distilled water. This
184 blank allowed the correction of the absorbance data considering the intrinsic optical density of the samples.
185 After preparation, the plate was incubated at room temperature inside the microplate reader (Synergy HT,

186 BioTek Instruments, United States) and the absorbance was read at a wavelength of 700 nm. The FRAP value
187 of LJRE was measured in triplicate and the antioxidant activity is reported as average concentration of TE
188 (μmol TE/g of extract DW) with the corresponding SEM.

189 1.5.3. 2,2-difenil-1-picrilhidrazilo (DPPH) radical scavenging activity assay:

190 The concentration of LJRE that scavenges the half of DPPH radical concentration (IC50 DPPH) was determined
191 by the microplate assay described by Kenny, Brunton, and Smyth (2015) with minor modifications. The IC50
192 DPPH for LJRE was calculated by interpolation in the corresponding Hill's dose-response equation (Chen et
193 al.,2013). The determination was carried out in triplicate and the antioxidant activity is reported as average
194 IC50 DPPH of the extract (μg/mL) with the corresponding SEM.

195 1.4.4. Ferrous Iron Chelating Activity assay (FICA):

196 The chelating activity of LJRE on iron (II) ion was assessed according to the procedure reported by (Santos et
197 al. 2017) with minor modifications. An iron (II) chloride solution with a concentration of 0.3 mM was used as
198 iron (II) source, and the ethylenediaminetetraacetic acid (EDTA) linearity range was fixed to 25-100 μM. The
199 chelating activity of LJRE was measured in triplicate and the FICA value is reported as average concentration
200 of EDTA equivalents (EDTAE) (μmol EDTAE/g of extract DW) with the corresponding SEM.

201 2. *Ex vivo models*

202 2.1. Animals and ethical approval

203 All experimental procedures involving animals were approved by the Institutional Committee for the Care and
204 Use of Animals (CICUA) of the University of Costa Rica under approval number CICUA-112-17. All methods
205 were carried out in accordance with institutional guidelines and national regulations governing the care and
206 use of laboratory animals.

207 Hsd:ICR (CD-1®) mice of both sexes (8–10 weeks old) were obtained from the Animal Facility of the Biological
208 Assays Laboratory (LEBi), University of Costa Rica. Animals were maintained under standardized housing
209 conditions with controlled temperature, humidity, and a 12 h light/dark cycle. Food and water were provided
210 *ad libitum*, except for a 12 h fasting period prior to tissue collection. Animals were euthanized by cervical
211 dislocation prior to tissue extraction.

212 2.2. Preparation of intestinal tissue for organ bath experiments

Intestinal segments were isolated according to the Magnus method. After excision, the intestine was placed in oxygenated nutrient solution in a Petri dish. From each intestinal segment, three sections of approximately 1 cm in length were prepared.

Each segment was mounted in an individual organ bath chamber containing 20 mL of nutrient solution. Tissue fixation followed a modification of the method described by Obembe et al. Instead of conventional suture needles, a straight needle and cotton thread were used to secure both ends of the intestinal segment. One end was attached to a force transducer, and the other end to a fixed support inside the organ bath chamber.

The bathing solution was maintained at 36 ± 2 °C through water recirculation from a digital water bath (Radnoti). Continuous oxygenation was provided using carbogen (95% O_2 and 5% CO_2).

Nutrient solution: Krebs–Henseleit nutrient solution was used as the physiological medium in all organ bath experiments. The solution contained (mmol/L): NaCl 118; KCl 4.7; $CaCl_2$ 2.5; $NaHCO_3$ 25; KH_2PO_4 1.2; $MgSO_4$ 1.2; anhydrous glucose 11.1.

2.3. Concentration–Response Curves

The experimental conditions, including the use of the ileum and an initial resting tension of 0.2 g, were established based on preliminary optimization experiments (unpublished data). These experiments also determined the appropriate concentration ranges for acetylcholine (ACh) and the aqueous extracts. Ileum segments were mounted in organ baths and allowed to equilibrate for approximately 15 min before each experiment. After each assay, the tissues were washed three times, and the protocol was repeated at least once using the same tissue when an adequate response was obtained; otherwise, the ileum segment was replaced.

2.3.1. Acetylcholine

Acetylcholine (ACh) was tested over a concentration of 1×10^{-10} - 3×10^{-5} mol/L in the *ex vivo* mouse ileum assay. The extracts (0.1 or 1.0 mg/mL) were added to separate organ baths and incubated for 10 min before cumulative additions of ACh were made at 3-min intervals to generate concentration–response curves.

2.3.2. Aqueous Rhizome Extracts

Preliminary optimization experiments were conducted to determine the appropriate concentration range for each extract. Both extracts were initially evaluated over a concentration range of 1×10^{-4} to 30 mg/mL, which

was progressively expanded until well-defined sigmoidal concentration–response curves with satisfactory goodness-of-fit (R^2) were obtained. Cumulative additions of the extract solutions were then made at 3-min intervals to generate concentration–response curves.

3. *In Vivo* Models

For the evaluation of acute oral toxicity, certified nulliparous female HsdBrlHan:WIST rats were used, whereas the antispasmodic and antidiarrheal activities were evaluated using CD-1® IGS mice (Crl:CD1(ICR)). All animals were bred and maintained at the animal facility of the Biological Assays Laboratory, University of Costa Rica.

3.1. Acute Oral Toxicity

Acute oral toxicity was evaluated according to OECD Guideline 423 (Acute Toxic Class Method). Fifteen certified nulliparous female HsdBrlHan: WIST rats (8–12 weeks old), bred at the Animal Facility of the Biological Assays Laboratory, University of Costa Rica, were used. Animals were acclimatized for 5 days and fasted for 12 h before treatment.

The aqueous rhizome extracts were administered by intragastric gavage following the sequential dosing procedure described in OECD 423. An initial dose of 300 mg/kg body weight was administered, followed by a second group at the same dose. As no mortality or treatment-related clinical signs were observed, two additional groups received 2,000 mg/kg body weight. Animals were monitored for clinical signs during the first 4 h after dosing and daily for 14 days. Gross necropsies were performed on representative control and treated animals at the end of the study.

3.2. Gastrointestinal Motility

Gastrointestinal motility was evaluated *in vivo* in male Hsd:ICR (CD-1®) mice aged 5-6 weeks using charcoal meal intestinal transit assay (de Oliveira et al. 2008). Animals were allocated to treatment groups (n=5 per group). Loperamide (1 mg/kg) and atropine sulfate (2 mg/kg) were administered intraperitoneally as positive controls, whereas saline solution was administered by oral gavage as the negative control. The experimental group received LJRE (2.000 mg/kg) by oral gavage.

Thirty minutes after treatment administration, each mouse received an oral dose of activated charcoal (10% w/v) suspended in 1.5 % agar, at a volume of 0.1 mL per 10 g of body weight. Thirty minutes after charcoal administration, the animals were euthanized by cervical dislocation. A laparotomy was performed, and the gastrointestinal tract was carefully removed from the stomach to the cecum. The distance traveled by the charcoal marker was measured from the pylorus to the most distal intestinal segment containing at least 1 cm of continuous charcoal. The total length of the small intestine was also measured.

Intestinal transit was expressed as the percentage of the total intestinal length traveled by the marker using the following equation:

Intestinal transit (%) = (Distance traveled by the marker / Total intestinal length) ×100

Data were expressed as mean ± SEM. The percentage inhibition of gastrointestinal transit was calculated relative to the control group using the following equation:

$$\text{Inhibition of gastrointestinal transit } (\%) = \frac{\text{Control transit} - \text{Treatment transit}}{\text{Control transit}} \times 100$$

3.3. Antidiarrheal Activity

Antidiarrheal activity was evaluated in male Hsd:ICR (CD-1®) mice (5–6 weeks old) using a modified method described by Adebayo et al. The dose of castor oil used to induce diarrhea (18.9 g/kg) was determined previously in a pilot study.

The aqueous rhizome extracts were administered orally at the selected doses prior to castor oil administration. Loperamide (10 mg/kg body weight) was used as the positive control, whereas saline solution (10 mL/kg) served as the negative control. Each treatment group included 4–5 animals. Following castor oil administration, animals were housed individually in cages fitted with wire mesh floors to prevent coprophagy, and the number of liquid fecal droppings was recorded over a 24-h period. Results were expressed as mean ± SEM.

Statistical analysis

For the *ex vivo* assays evaluating the effects of LJRE on acetylcholine-induced contractions in isolated mouse ileum, normality and homogeneity of variances were assessed using the Shapiro–Wilk and Levene's tests,

respectively. Data met the assumption of normality but showed heterogeneity of variances; therefore, differences among groups were analyzed using Welch's one-way ANOVA followed by the Games–Howell post hoc test for multiple comparisons.

For the *in vivo* assays, normality and homogeneity of variances were assessed using the Shapiro–Wilk and Levene's tests, respectively. As both assumptions were met, differences among groups were analyzed using one-way ANOVA followed by Dunnett's post hoc test. Data are presented as mean ± standard error of the mean (SEM), and differences were considered statistically significant at $p < 0.05$.

Results

Phytochemical screening was performed to identify the main groups of secondary metabolites present in *L. japurensis* rhizome extract (LJRE). The qualitative findings are presented in Fig. 1 and Table 1.

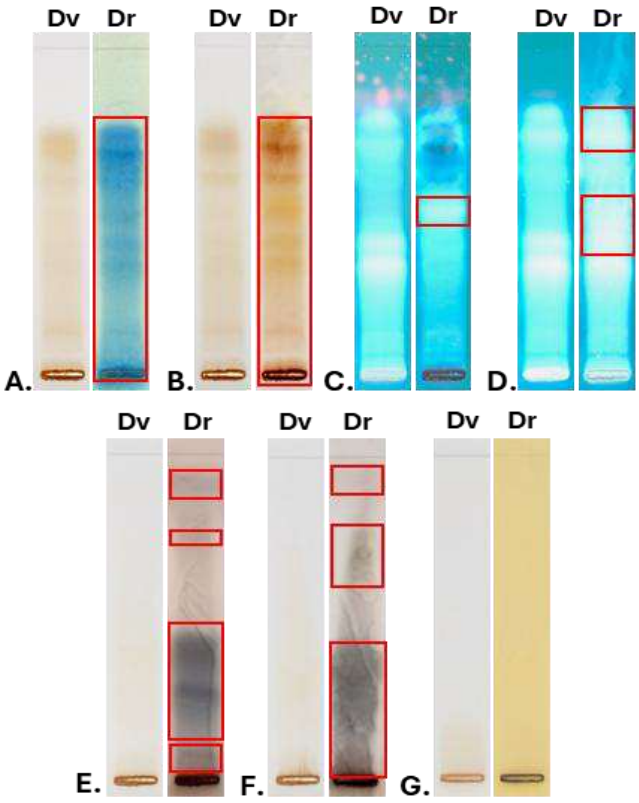


Fig. 1 *TLC chromatograms for the detection of secondary metabolite groups in Lomariopsis japurensis rhizome*

Note. **A**. General phenolics (derivatization with potassium ferricyanide and ferric chloride reagents; observed under visible light), **B**. Flavonoids and quinones (derivatization with potassium hydroxide reagent; observed under visible light), **C**. Coumarins

(derivatization with potassium hydroxide reagent; observed under UV light with a wavelength of 366 nm) **D**. Flavonoids (derivatization with aluminum chloride reagent; observed under UV light with wavelength of 366 nm), **E**. General terpenoids (derivatization with anisaldehyde sulfuric acid reagent; observed under visible light), **F**. Triterpenes and sterols (derivatization with Libermann-Burchard's reagent; observed under visible light), **G**. Alkaloids (derivatization with Dragendorff's reagent; observed under visible light). Dv: Visualization after plate development, Dr: Visualization after plate derivatization. Major positive changes are shown inside red boxes.*

Table 1. Qualitative detection of tannins and saponins in *Lomariopsis japurensis* rhizome extract (LJRE).

Phytochemical group	**Qualitative test**	**Result**
Tannins	Ferric chloride test	+
Tannins	Gelatin–salt test	+
Saponins	Foam test	+

LJRE was characterized in terms of its total phenolic content, total flavonoid content, and antioxidant activity using complementary *in vitro* assays (Table 2).

Table 2. Total phenolic content (TPC), total flavonoid content (TFC), and *in vitro* antioxidant activity of *Lomariopsis japurensis* rhizome extract (LJRE).

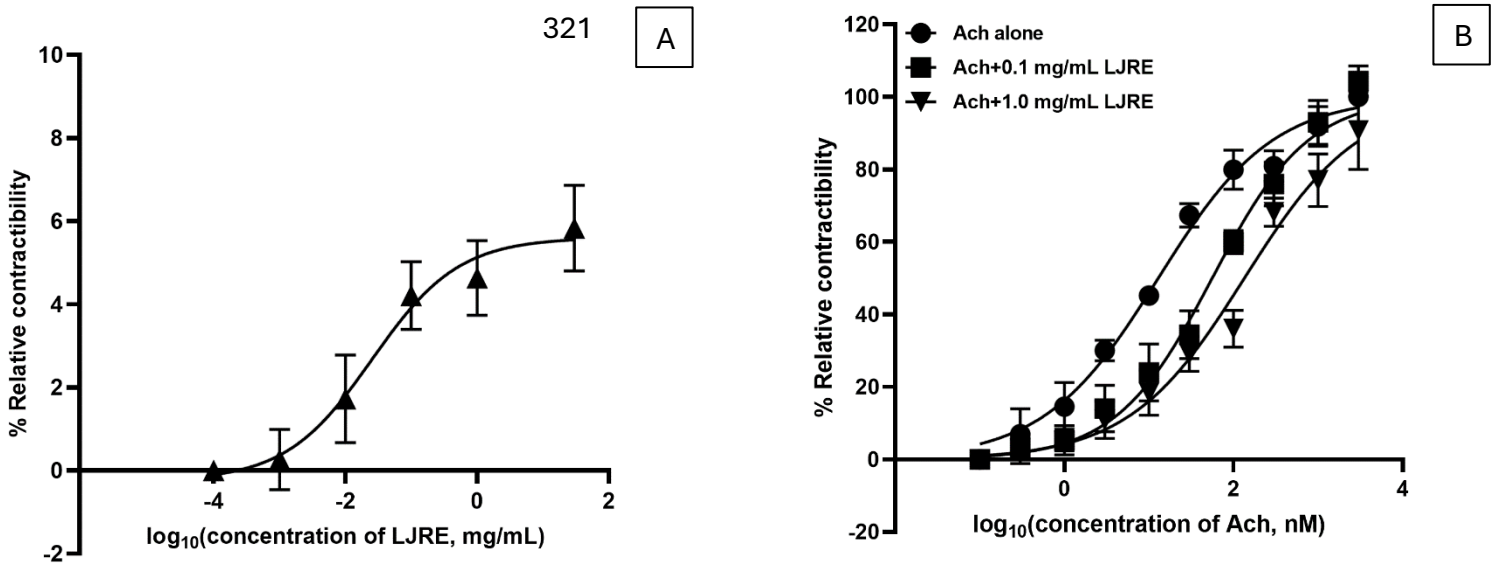
Measured parameter	**Average ± SEM**
TPC (mg GAE/g DW)	115.27 ± 0.88
TFC (mg QE/g DW)	318.57 ± 3.30
ORAC (mmol TE/g DW)	3.29 ± 0.08
FRAP (mmol TE/g DW)	0.90 ± 0.02
IC_{50} DPPH (µg/mL)	15.21 ± 0.65
FICA (µmol EDTAE/g DW)	48.09 ± 0.22

318 The effects of LJRE on acetylcholine-induced contractions were evaluated in isolated mouse ileum to assess

319 changes in tissue responsiveness to cholinergic stimulation (Fig. 2).

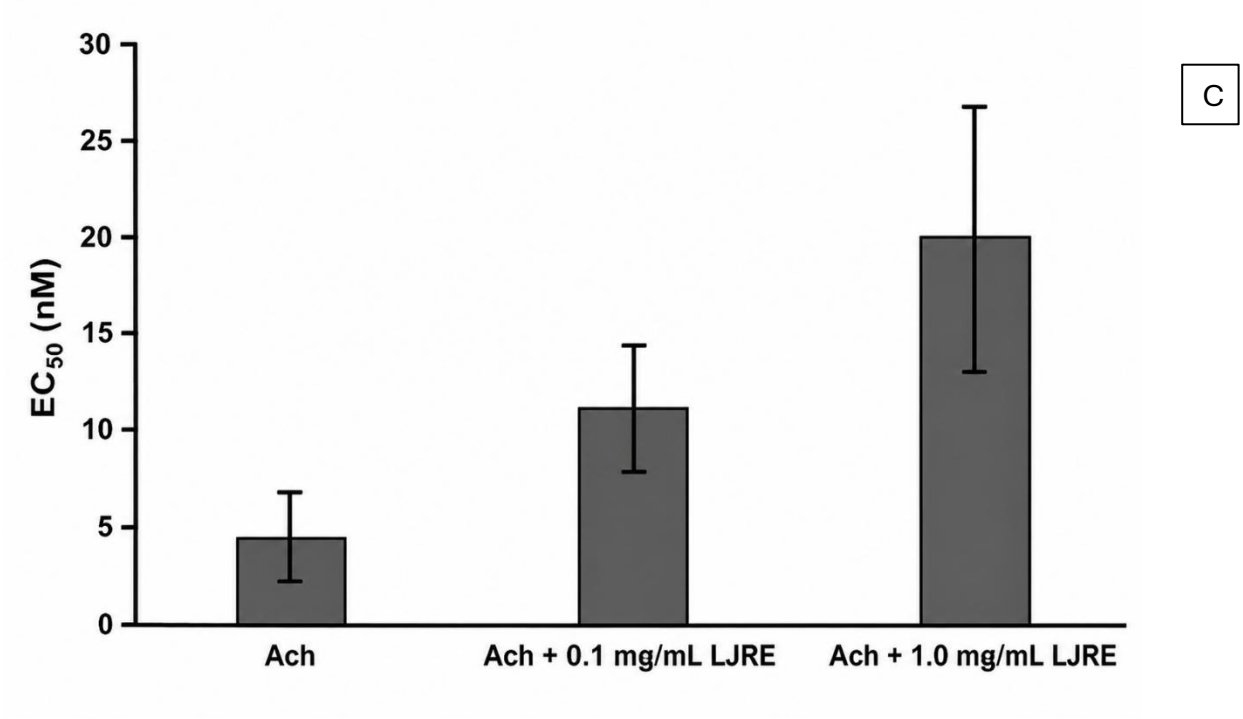
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Fig. 2. Effects of *Lomariopsis japurensis* rhizome extract on acetylcholine-induced contractions in isolated mouse ileum.

The concentration–response curves showed goodness-of-fit values of $R^2 > 0.985$. The maximum contractile response induced by the LJRE was 5.83 ± 1.03%, corresponding to a concentration of 30 mg/mL. EC_{50} values (mean ± SEM) for acetylcholine were 4.65 ± 1.92 nM (ACh alone), 11.01 ± 2.66 nM (ACh + 0.1 mg/mL LJRE), and 20.15 ± 5.77 nM (ACh + 1.0 mg/mL LJRE). EC_{50} values differed significantly among groups (Welch's one-way ANOVA, $p < 0.05$). The Games-Howell post hoc test showed that preincubation with 1.0 mg/mL LJRE significantly increased the EC_{50} of ACh compared with ACh alone ($p < 0.05$), whereas no significant difference was observed with 0.1 mg/mL LJRE. Data are presented as mean ± SEM (n = 5), except for the concentration–response curve of the LJRE (**A**), where n = 2.

The acute oral toxicity of LJRE was evaluated in rats, with observations recorded over 14 days following administration (Table 3).

Table 3. Acute oral toxicity of *Lomariopsis japurensis* rhizome extract (LJRE) in rats.

Parameter	Control	LJRE 300 mg/kg	LJRE 2,000 mg/kg
Mortality	0/3	0/6	0/6
Clinical signs of toxicity	None	None	None
Changes in general behavior	None	None	None
Changes in food/water intake	None	None	None
Abnormal body-weight changes	None	None	None
Macroscopic alterations at necropsy	None	None	None

Note. LJRE: Animals were monitored for 14 days after oral administration. Acute oral toxicity was assessed according to OECD Guideline 423.

The effect of LJRE on intestinal motility was assessed by measuring gastrointestinal transit in mice (Fig. 3).

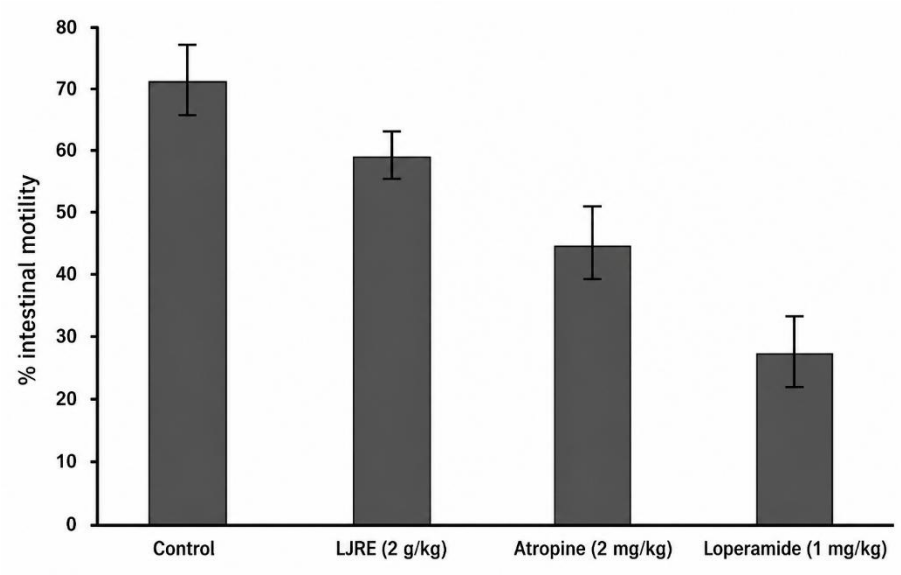


Fig. 3. Effect of *Lomariopsis japurensis* rhizome extract (LJRE) on intestinal motility in mice.

Intestinal motility was evaluated using the charcoal meal gastrointestinal transit test. Mice received saline (control), LJRE (2.0 g/kg), atropine (2.0 mg/kg), or loperamide (1.0 mg/kg). Gastrointestinal transit was 71.30 ± 4.87% in the control group and was significantly reduced to 59.28 ± 3.32% following treatment with LJRE, corresponding to a 16.9% inhibition relative to the control. Atropine and loperamide reduced gastrointestinal transit to 44.36 ± 5.60% and 27.50 ± 5.11%, corresponding to inhibitions of 37.8% and 61.4%, respectively. Data are presented as mean ± SEM (n = 5). One-way ANOVA showed significant differences among groups ($p < 0.001$), and Dunnett's post hoc test showed that LJRE, atropine, and loperamide significantly reduced gastrointestinal transit compared with the control group ($p < 0.001$).

The antidiarrheal activity of LJRE was evaluated in mice using the castor oil-induced diarrhea model (Fig. 4).

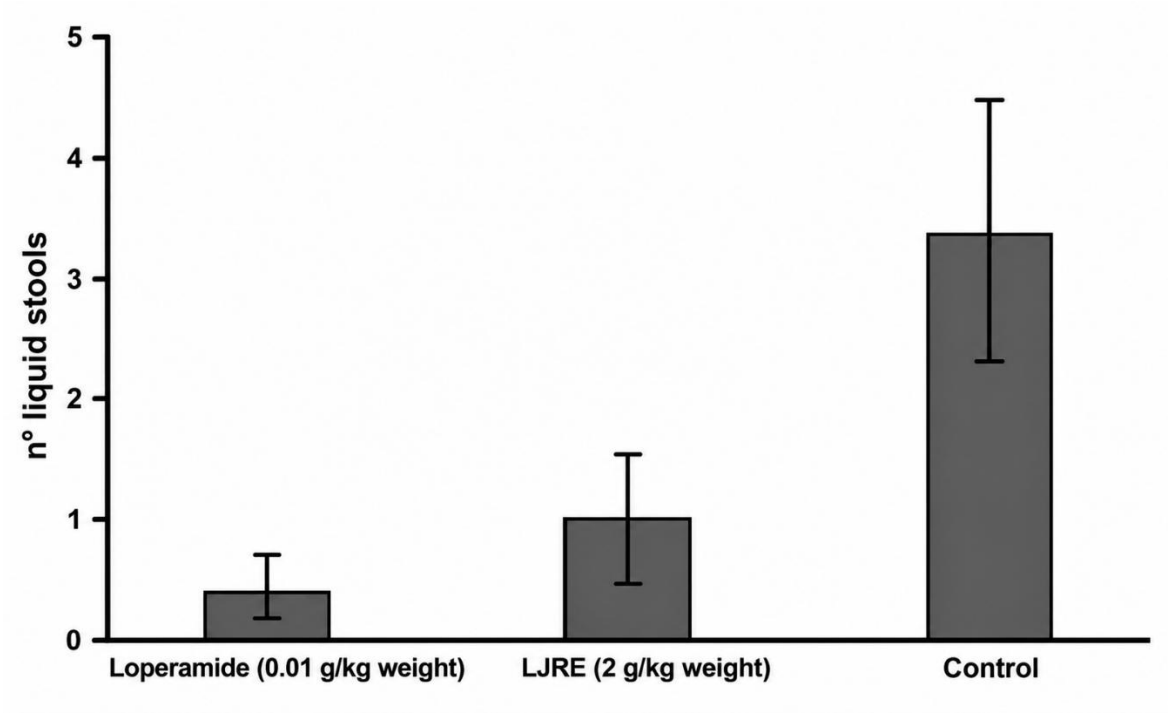


Fig. 4. Antidiarrheal effect of *Lomariopsis japurensis* rhizome extract (LJRE) in the castor oil-induced diarrhea model.

The number of liquid fecal droppings was recorded over 24 h in Hsd:ICR mice receiving castor oil (18.9 g/kg). Castor oil, saline (negative control), LJRE (2.000 mg/kg), and loperamide (10 mg/kg) were administered by oral gavage. The negative control presented 3.4 ± 1.0 liquid fecal droppings, whereas treatment with LJRE (2,000 mg/kg) significantly reduced this number to 1.0 ± 0.55, corresponding to a 70.6% reduction relative to the negative control. Loperamide (10 mg/kg) reduced the number of liquid fecal droppings to 0.4 ± 0.24, corresponding to an 88.2% reduction. Data are presented as mean ± SEM (n =5). One-way ANOVA showed significant differences among groups ($p < 0.05$), and Dunnett's post hoc test showed that both LJRE and loperamide significantly reduced the number of liquid fecal droppings compared with the negative control ($p < 0.05$).

Discussion:

As shown in Figure 1, HPTLC analysis demonstrated that LJRE contains several groups of secondary metabolites, including phenolics and terpenoids, particularly flavonoids, coumarins, triterpenoids and sterols. No alkaloids were detected using Dragendorff's reagent under the experimental conditions employed and alkaloids. In addition, conventional qualitative phytochemical screening revealed the presence of tannins and saponins (Table 1). It is difficult to contrast these findings with other species of the genus *Lomariopsis* because of the limited phytochemical information available. However, in *L. guineensis*, flavonoids, steroids, and tannins have been reported, providing a phytochemical precedent within the genus for several of the metabolite

groups detected in *L. japurensis* (Al-Tannak et al. 2023, Mih et al. 2017). In contrast, saponins were not detected in *L. guineensis* (Mih et al. 2017), suggesting possible differences in the phytochemical composition between these species.

Expanding the comparison to species of the Polypodiaceae family, *Polypodium vulgare* has been recognized as a source of polyphenols, including flavonoids and phenolic acids; and sterols (Farràs et al. 2023). *Nephrolepis cordifolia* has been identified as a source of polyphenols (mainly flavonoids and tannins), triterpenoids, sterols, and alkaloids (Gonçalves dos Santos et al. 2024). These data, except for the absence of alkaloids and the presence of coumarins, suggest that the main metabolite groups present in *Lomariopsis japurensis* are consistent with the information reported for other taxonomic related ferns. Interestingly, flavonoids, coumarins, triterpenes, and sterols are groups of compounds considered as sources of spasmolytics (Amor et al. 2005, Gomes et al. 2005, Sharifi-Rad et al. 2021). Therefore, it is important to have an in-depth approach into these metabolite categories to determine the identity of the most important bioactive compounds responsible for the observed smooth muscle relaxant effect.

On the other hand, polyphenols are widely known for their antioxidant activity. Flavonoids are one of the most important kinds of compounds in terms of this effect. They can exert antioxidant effects through several chemical mechanisms including hydrogen atom transfer (HAT), single electron transfer (SET), and chelation of transition metals (Chen et al. 2022, Kumar and Pandey 2013). Using *in vitro* assays based on different chemical principles is important to evaluate the contribution of each of these mechanisms to the observed antioxidant effects of substances or plant extracts. ORAC measures the ability of the extracts to stabilize free radicals due to HAT mechanism, FRAP allows to assess the capability of the extracts to reduce free radicals by SET mechanism, DPPH assay enables the evaluation of the antioxidant activity by the combination of both mechanisms, HAT and SET (Schaich et al. 2015), and FICA assay evaluates the chelation capability of the extracts on transition metals that can be implicated in Fenton and Haber-Weiss reactions as the case of iron (II) (Kehrer 2000, Santos et al. 2017). The formation of a complex between polyphenols and iron (II) allows a decline in the production of oxygen reactive species by these reactions that are implicated in oxidative stress in biological systems (Dai and Mumper 2010, Kehrer 2000).

As shown in Table 2, LJRE demonstrated high total phenolic content (TPC) and total flavonoid content (TFC), with values of 115.27 mg GAE/g and 318.57 mg QE/g, respectively. These values are noteworthy considering

403 those reported for herbs recognized as rich sources of polyphenols and antioxidants, such as rosemary and
404 sage (Embuscado, 2015). In fact, the TPC and TFC values of LJRE exceeded those reported by Bianchin et al.
405 (2020) for rosemary and sage ethanol extracts obtained by digestion with 80% (v/v) ethanol followed by
406 lyophilization. Those authors reported TPC values of 46.48 mg GAE/g and 41.61 mg GAE/g, and TFC values of
407 11.89 mg QE/g and 15.03 mg QE/g for rosemary and sage extracts, respectively (Bianchin et al. 2020).

408 Consistent with its high phenolic and flavonoid contents, LJRE also exhibited remarkable antioxidant activity
409 (Table 2). Its ORAC value was higher than those reported for rosemary and sage extracts obtained by
410 percolation with 50% and 70% (v/v) ethanol, respectively, followed by freeze-drying (Ninfali et al. 2009).
411 Likewise, the FRAP value of LJRE (0.90 mmol TE/g) was within the range reported for commercial rosemary
412 and green tea extracts (0.14–1.76 mmol TE/g), while its DPPH IC_{50} value (15.21 μg/mL) was also comparable
413 to those reported for the same commercial extracts (11.70–22.46 μg/mL) (Pires et al., 2017). Furthermore,
414 LJRE demonstrated a FICA value of 48.09 μmol EDTAE/g, which should be compared with the values reported
415 for *Calendula officinalis* methanolic extracts obtained by continuous percolation (58 μmol EDTAE/g) (Ak et al.
416 2020) and *Psidium guajava* leaf extracts produced by decoction with 70% (v/v) ethanol (Ruksiriwanich et al.
417 2022). Based on the high concentrations of phenolic compounds and flavonoids together with the results of
418 the different antioxidant assays, we propose that these compounds are major contributors to the antioxidant
419 activity of LJRE.

420 As illustrated in Figure 2, LJRE exhibited a dual pharmacological profile. When administered alone (Figure 2A),
421 the extract induced a weak contractile response, reaching less than 6% of the maximal contraction elicited by
422 ACh, indicating a very low intrinsic contractile activity. As shown in Figures 2B and 2C, preincubation with
423 LJRE significantly reduced the potency of ACh, indicating that higher concentrations of the neurotransmitter
424 were required to elicit the same contractile response. These findings demonstrate that LJRE decreased the
425 responsiveness of intestinal smooth muscle to cholinergic stimulation despite its weak direct contractile
426 activity, resulting in an overall attenuation of ACh-induced contractions (Zholos et al. 2024).

427 Since acetylcholine is the principal excitatory neurotransmitter regulating gastrointestinal smooth muscle
428 contraction, primarily through activation of M_3 muscarinic receptors, the present findings suggest that LJRE
429 modulates cholinergic-mediated intestinal contraction. However, because muscarinic receptor activation also
430 involves M_2-dependent modulation and multiple downstream signaling pathways, including TRPC4 activation

and Ca^{2+} influx, the precise molecular target cannot be identified from the present data. Considering that LJRE is a complex mixture of bioactive phytochemical constituents, different compounds may account for its weak intrinsic contractile activity and its inhibitory effect on ACh-induced contractions. Therefore, although modulation of muscarinic receptors represents one plausible mechanism, the involvement of additional molecular targets regulating intestinal smooth muscle contraction cannot be excluded (Guzman et al. 2026, Zholos et al. 2024).

As shown in Table 3, no mortality, treatment-related clinical signs, relevant alterations in general health status, or macroscopic abnormalities in the organs and tissues examined at necropsy were observed following administration of LJRE at doses up to 2,000 mg/kg. According to the principles established for acute oral toxicity assessment, the absence of mortality and evident signs of toxicity at the 2,000 mg/kg limit dose is consistent with a low acute toxicity profile (OECD, 2001).

As shown in Figure 3, LJRE reduced gastrointestinal transit compared with the control group, although its inhibitory effect was less pronounced than those observed with atropine and loperamide. This finding supports the antidiarrheal potential of LJRE and suggests that its activity may be associated, at least in part, with a reduction in intestinal propulsion. Decreased gastrointestinal transit increases the time available for intestinal fluid and electrolyte absorption and may consequently contribute to a reduction in the frequency and liquidity of stools (Hammesso et al. 2025, Marasco et al. 2026).

Consistent with these findings, Figure 4 shows that LJRE reduced the number of liquid stools compared with the control group. This effect provides additional *in vivo* evidence of the antidiarrheal activity of the extract and is consistent with the reduction in gastrointestinal motility observed in Figure 3. The lower frequency of liquid stools may therefore be related, at least in part, to slower intestinal propulsion, which prolongs the contact time between luminal contents and the intestinal mucosa and favors fluid and electrolyte absorption (Lenti et al. 2025, Marasco et al. 2026).

Taken together, the *ex vivo* and *in vivo* findings support a consistent pharmacological effect of LJRE on intestinal function. The reduced responsiveness of isolated ileum to ACh (Figure 2), together with the decrease in gastrointestinal transit (Figure 3) and the lower number of liquid stools (Figure 4), suggests that attenuation of intestinal smooth muscle contractility and propulsion may contribute to the antidiarrheal activity of LJRE.

Similar associations between reduced gastrointestinal motility and antidiarrheal activity have recently been reported for plant-derived extracts (Noor and Terefe, 2025).

Notably, LJRE reduced liquid stools by 70.6%, whereas gastrointestinal transit was inhibited by 16.9% relative to the control. Although these percentages represent different experimental endpoints and cannot be directly compared, the marked reduction in liquid stools suggests that inhibition of intestinal motility alone may not fully explain the antidiarrheal effect. Additional mechanisms, potentially involving intestinal secretion or water and electrolyte transport, may therefore contribute to the observed activity (Okorie et al. 2025).

The phytochemical composition of LJRE may provide a basis for these pharmacological effects. Flavonoids may influence intestinal contractility through modulation of Ca^{2+} and K^{+} channels, whereas some coumarins have been shown to attenuate intestinal motility through inhibition of TMEM16A, a Ca^{2+}-activated Cl^{-} channel involved in the regulation of intestinal motor activity (Chomentowski et al. 2025, Zhu et al. 2025). Tannins may also contribute to the antidiarrheal activity of LJRE, as tannin-containing plant extracts have been associated with reductions in stool frequency and water content in castor oil-induced diarrhea models. These effects may involve astringent actions on the intestinal mucosa and reductions in intestinal secretion and fluid loss (Hoque et al. 2025, Hounsa et al. 2026).

Saponins detected in LJRE may also contribute to its antidiarrheal activity. Dietary alfalfa saponins have been reported to decrease diarrhea and modify gastrointestinal conditions in weaned piglets, whereas saponin-rich extracts from *Panax notoginseng* have been associated with improvement of intestinal mucosal integrity and modulation of inflammatory responses and intestinal microbiota. These findings suggest that saponin-containing preparations may influence intestinal function through mechanisms other than direct modulation of motility (Fan et al. 2024, Zhao et al. 2026). However, the specific contribution of saponins to the antidiarrheal activity of LJRE remains to be established.

Overall, these findings suggest that the antidiarrheal effect of LJRE may result from the combined influence of several mechanisms rather than from inhibition of intestinal motility alone. The modulation of intestinal contractility observed *ex vivo*, together with the reduction in gastrointestinal transit and liquid stools *in vivo*, supports the traditional use of *L. japurensis* for diarrhea. However, further studies are required to identify the bioactive constituents responsible for these effects and to determine their specific mechanisms of action.

Conclusions:

Lomariopsis japurensis rhizome extract (LJRE) showed antispasmodic and antidiarrheal effects in the experimental models evaluated. *Ex vivo*, LJRE reduced the responsiveness of the isolated ileum to acetylcholine, as evidenced by the increased EC_{50}, while in vivo it decreased gastrointestinal transit at a dose 2000 mg/kg. LJRE markedly reduced the number of liquid stools in the castor oil-induced diarrhea model. The greater reduction in liquid stools relative to the inhibition of gastrointestinal transit suggests that mechanisms other than reduced intestinal motility may contribute to its antidiarrheal activity.

Phytochemical screening revealed several classes of secondary metabolites, including flavonoids, coumarins, tannins, and saponins, which may contribute to the observed pharmacological effects. LJRE also exhibited antioxidant activity *in vitro* and showed no evidence of acute oral toxicity under the experimental conditions evaluated. Overall, these findings provide experimental support for the traditional use of *L. japurensis* for gastrointestinal disorders, including diarrhea. Further studies are required to identify the bioactive constituents and clarify the mechanisms underlying its antispasmodic and antidiarrheal activities.

Limitations

This study has several limitations. First, the phytochemical characterization was primarily qualitative and group-based, and the individual compounds responsible for the observed biological effects were not isolated or identified. Second, the antioxidant activity was evaluated using *in vitro* chemical assays, which do not necessarily reflect antioxidant effects under physiological conditions. Third, the intestinal pharmacological evaluation was performed using isolated ileum preparations; therefore, the molecular mechanisms underlying the modulation of acetylcholine-induced contractions remain to be established. Finally, although LJRE showed effects on gastrointestinal transit and castor oil-induced diarrhea in mice, these findings are based on experimental animal models and cannot be directly extrapolated to clinical efficacy in humans.

Recommendations

Further studies should focus on the isolation and structural identification of the bioactive constituents of LJRE and on determining their contribution to its antispasmodic and antidiarrheal effects. Mechanistic studies are also needed to clarify the molecular pathways involved in the modulation of intestinal smooth muscle

contractility and to investigate whether the marked antidiarrheal effect involves additional mechanisms related to intestinal secretion and water and electrolyte transport. The antioxidant activity should be further evaluated in biological models to determine its potential physiological relevance. In addition, subacute and chronic toxicity studies are recommended to expand the safety profile of LJRE. Finally, further preclinical studies using complementary models are required before considering the potential clinical application of *L. japurensis*.

Abbreviations

ACh: Acetylcholine

CICUA: Institutional Committee for the Care and Use of Animals

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

DW: Dry weight

EC_{50}: Half-maximal effective concentration

EDTA: Ethylenediaminetetraacetic acid

EDTAE: Ethylenediaminetetraacetic acid equivalents

FICA: Ferrous iron chelating activity

FRAP: Ferric reducing antioxidant power

GAE: Gallic acid equivalents

HAT: Hydrogen atom transfer

HPTLC: High-performance thin-layer chromatography

IC_{50}: Half-maximal inhibitory concentration

LEBi: Biological Assays Laboratory

LJRE: *Lomariopsis japurensis* rhizome extract

OECD: Organisation for Economic Co-operation and Development

ORAC: Oxygen radical absorbance capacity

QE: Quercetin equivalents

SEM: Standard error of the mean

SET: Single electron transfer

TE: Trolox equivalents

TFC: Total flavonoid content

TLC: Thin-layer chromatography

TPC: Total phenolic content

UV: Ultraviolet

Declarations

Ethics approval and consent to participate

All experimental procedures involving laboratory animals were reviewed and approved by the Institutional Committee for the Care and Use of Animals (CICUA) of the University of Costa Rica (approval No. CICUA-112-17). Consent to participate was not applicable.

The collection of *Lomariopsis japurensis* plant material was conducted under authorization from the National System of Conservation Areas (SINAC), Costa Rica, under scientific collection permit No. SINAC-ACC-019-2018. Consent to participate was not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SLHS: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Project administration, Writing original draft, Writing review & editing. FD: Methodology, Investigation, Formal analysis, review & editing. NTWH: Methodology, Validation, Supervision, review & editing. SG: Methodology, Validation, Supervision, review & editing. OH: Conceptualization, Methodology, review & editing. All authors read and approved the final manuscript.

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